

Localization of Immunogenic Domains in the Human Immunodeficiency Virus Type 2 Envelope

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Highly immunogenic domains have not yet been defined in the extracellular protein of the human immunodeficiency virus type 2 (HIV-2) envelope. In this study, six contiguous segments covering the entire HIV-2_{ST} envelope were amplified and cloned into a bacterial expression vector to localize the relative immunogenic reactivity of different regions of the molecule by Western blot (immunoblot) analysis. Our results demonstrate that the extracellular protein of the HIV-2 envelope contains highly immunogenic epitopes with potential value as type-specific markers for HIV-2 infection.

Two types of viruses in the human immunodeficiency virus (HIV) family are known: HIV type 1 (HIV-1) and HIV-2. Both types of HIV have been etiologically linked to AIDS (3, 14, 35). While HIV-1 infection has resulted in a worldwide epidemic of AIDS, HIV-2 infection is, so far, endemic to West Africa (17, 22) with only occasional identification in Europe (5) and North America (25, 37).

HIV-2 was first noted when serum samples collected from West Africa were found to lack antibodies to the HIV-1 *env* protein but had readily detectable antibodies to the HIV-1 *gag* protein and to the simian immunodeficiency virus (SIV) *env* protein (2, 22). HIV-2 was subsequently isolated and grown in continuous cultures (7). Moreover, sequence comparison, particularly in the envelope region, demonstrated that HIV-2 is more related to SIV than to HIV-1 (8, 20, 43). Envelope glycoprotein sequence comparison among immunodeficiency virus strains shows that HIV-2 strains have approximately 35% homology with HIV-1 and 70% homology with SIV_{mac} in the amino acid sequence and 57% similarity with HIV-1 and 72% similarity with SIV_{mac} in the nucleic acid sequence (Table 1).

In vitro, HIV-2 shares several biological features with HIV-1: the ability to bind CD4 molecules, T-cell and mononuclear cell tropism, syncytium formation in CD4-positive cells, cytopathicity to T cells and other mononuclear cells, and a propensity for genetic changes (13, 32, 38, 49). In vivo, HIV-2 has been linked to clinical cases of AIDS (25, 36) but appears to be less virulent than HIV-1 (30, 35).

The amino terminus of the HIV-2 envelope transmembrane glycoprotein is the only region yet shown to be highly immunoreactive with the majority of HIV-2-positive sera (42, 50). In a study by Schulz et al. (42), the recombinant protein expressed by the plasmid pHIV-2/1 spanning amino acids (aa) 555 to 761 of the HIV-2_{ROD} envelope transmembrane protein was found to react with 17 of 18 HIV-2-positive serum samples. Similarly, Zuber et al. (50), on the basis of the high immunoreactivity of the recombinant proteins expressed by plasmid pMZ921, pMZ996, or pMZ997 spanning aa 537 to 707 of HIV-2_{NIH-z}, also concluded that the amino terminus of the transmembrane protein gp36 contains highly immunogenic epitopes. These findings are in contrast with those reported for the HIV-1 envelope protein

in that highly immunogenic epitopes in both the transmembrane glycoprotein gp41 (6, 9) and the extracellular glycoprotein gp120 (28, 29, 34) have been identified.

The present study was designed to survey different fragments of the HIV-2 envelope, with emphasis on whether immunogenic epitopes could also be found in other parts of the envelope, particularly in the extracellular glycoprotein of HIV-2. Our results demonstrate that the extracellular domain of the HIV-2 envelope also contains highly immunogenic epitopes in a region comparable to the third hypervariable domain of the HIV-1 gp120 in addition to the previously recognized region in the amino terminus of HIV-2 transmembrane glycoprotein.

To localize immunogenic domains in the HIV-2 envelope, we adopted the method of polymerase chain reaction (PCR) (39) to amplify DNA fragments from the plasmid pJSP4-27/H6 (27), which contains the 3' partial sequence of the provirus including the entire envelope coding sequence of the ST strain of HIV-2 (HIV-2_{ST}). HIV-2_{ST} was originally isolated from an asymptomatic Senegalese carrier (26). In vitro, HIV-2_{ST} showed minimal cytopathicity compared with other HIV strains (26). The *env* protein sequence of HIV-2_{ST}, however, shares a high degree of conservation with those of other HIV-2 strains, including ROD (7, 18), NIH-z (49), SBL-6669/ISY (1, 11, 12), GH1 (19), and BEN (24), as shown in Table 1.

TABLE 1. Homology of the envelope amino acid and nucleic acid sequences among the HIV-2 viral strains and with HIV-1 and SIV

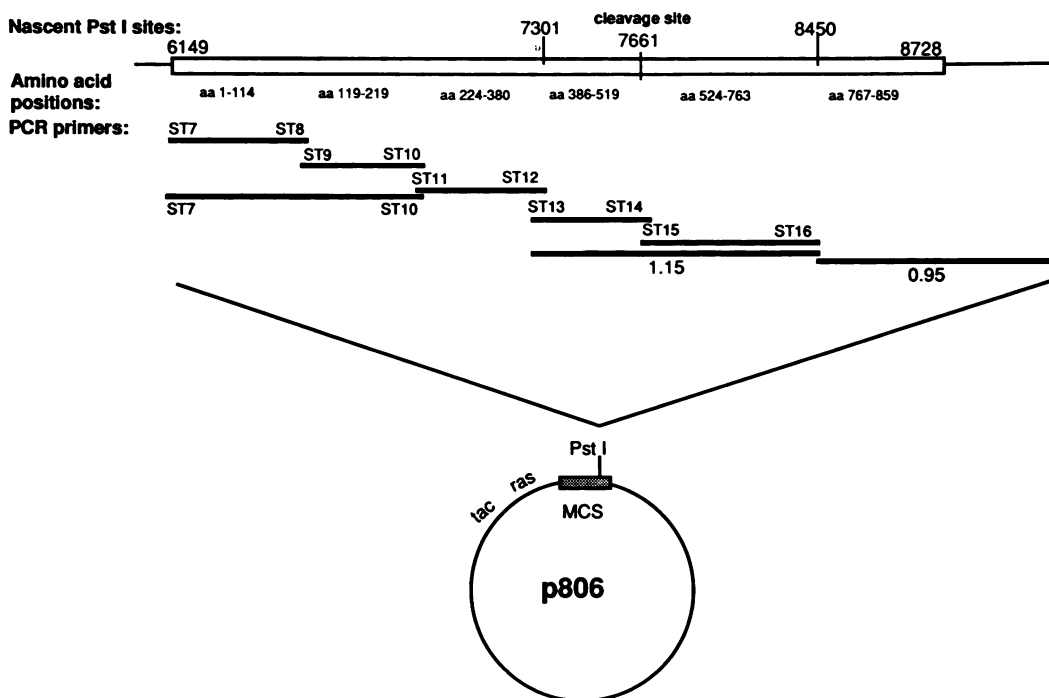
Virus	% Homology with ^a :							
	HXB2 ^b	ROD	NIH-z	SBL/ISY	ST	GH1	BEN	SIV
HXB2		35.2	36.1	35.5	35.4	37.3	34.4	32.2
ROD	55.8		81.3	79.9	81.3	83.7	77.9	70.9
NIH-z	57.4	88.3		79.9	83.5	81.2	76.9	72.5
SBL/ISY	57.3	86.3	86.9		82.6	82.8	77.4	69.8
ST	56.9	87.3	87.3	87.8		87.7	79.4	72.0
GH1	57.5	88.4	86.5	86.6	90.5		82.4	74.6
BEN	56.8	84.8	84.3	83.2	84.6	87.3		68.9
SIV	57.9	70.0	73.7	73.0	72.6	72.7	69.6	

^a Amino acid and nucleic acid sequence homologies are shown in the top and bottom diagonal portions of the table, respectively.

^b HIV-1 strain.

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A. HIV-2_{ST} envelope



B. PCR primer sequences



FIG. 1. Cloning strategy for the expression clones using PCR (A); PCR primer pair sequences for clones ST7-8, ST9-10, ST11-12, ST13-14, and ST15-16 (B). In each primer, the *Pst*I site is underlined. PCR was performed for 40 cycles with *Taq* polymerase according to the manufacturer's specifications (Perkin-Elmer Cetus, Emeryville, Calif.) with a programmable thermal controller (PTC-100; MJ Research, Inc., Cambridge, Mass.). Briefly, 1 ng of the template was mixed with 1 μ g of each oligonucleotide primer. Forty cycles were performed; each cycle contained a 1-min denaturation step at 92°C, a 2-min annealing step at 5°C below the lower melting temperature of each oligonucleotide primer pair, and a 3-min polymerization step at 72°C. The amplified DNA was digested to completion with *Pst*I, gel purified, and cloned into the *Pst*I site of p806. The sequence at the junction between the 3' end of *v-ras*^H and the 5' end of the insert DNA was verified by the dideoxynucleotide chain termination sequencing method (41) with Sequenase version 2.0 (United States Biochemicals, Cleveland, Ohio).

To express the envelope recombinant proteins, the entire envelope coding sequence of HIV-2_{ST} was divided into six contiguous regions. These subregions were amplified and cloned into the bacterial expression vector p806 (Fig. 1A). The expression vector p806 is a derivative of pXVR (10) with 111 residual aa of *v-ras*^H at the 5' end of the multiple cloning

site. PCR primers (Fig. 1B) were designed so that the primers at each end of the amplified region contained a *Pst*I site and the insert would be in phase with the translational frame of the preceding *v-ras*^H (Fig. 1A). In addition, a translational stop codon was introduced near the 3' end of each amplified fragment to confine the final translational

TABLE 2. Seroreactivity of HIV-2_{ST} envelope recombinant proteins with HIV-2 serum samples from Senegal and HIV-1-positive serum samples from the United States

Serum sample type (n)	No. of samples (%) reactive with:						
	ST7-8	ST9-10	ST7-10	ST11-12	ST13-14	ST15-16	ST0.95
HIV-2 positive (48)	0 (0)	5 (10.4)	4 (8)	46 (95.8)	2 (4.2)	47 (97.9)	0 (0)
HIV-2 negative (22)	0	0	0	0	1	1	0
HIV-1 positive (20)	0	0	0	0	0	1	0

product. The oligonucleotide primers were synthesized by β -cyanoethyl phosphoramidite chemistry with a DNA synthesizer (Biotix, Danbury, Conn.).

The fragments generated by PCR were numbered according to the names of the oligonucleotide primers used. The six fragments generated are as follows: ST7-8 spanning the nucleotides (nt) 6142 to 6500, ST9-10 (nt 6495 to 6815), ST7-10 (nt 6142 to 6815), ST11-12 (nt 6810 to 7302), ST13-14 (nt 7297 to 7715), and ST15-16 (nt 7710 to 8451) (Fig. 1A). In addition, two *Pst*I fragments, ST1.15 and ST0.95, were isolated from pJSP4-27/H6 by gel purification and cloned into p806.

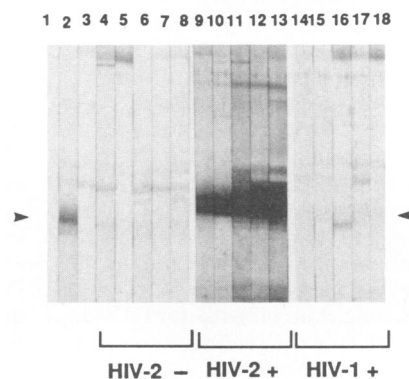
After cloning, the recombinant proteins were induced by 5 mM isopropyl- β -D-thiogalactopyranoside to the log-phase host *Escherichia coli* of either strain TG1 or strain JM109, both with a *lacI^q* genotype. The successful induction of fusion proteins was indicated by their expected sizes and immunoreactivities with a postimmune goat anti-*ras* serum (45, 48). The identities of the induced recombinant proteins were also verified by their nonreactivities against the preimmune goat serum (data not shown). Because of multiple nonspecific bands present in the bacterial lysate, partial purification was performed with recombinant proteins ST9-10, ST11-12, and ST15-16 for Western blot (immunoblot) analysis. The purification followed the procedure of Matsuda et al. (31). Supernatants from the extractions in 3 M and 8 M urea and the remaining pellet fraction were electrophoresed on a sodium dodecyl sulfate-12.5% polyacrylamide gel under reducing conditions to determine the sizes of the fusion proteins. The major fraction containing the solubilized recombinant protein was then blotted onto a nitrocellulose filter (BA85; Schleicher & Schuell, Keene, N.H.) and immunostained with a 1:1,000 dilution of preimmune or postimmune goat anti-*ras* serum (data not shown).

The immunoreactivity of each recombinant protein with human serum was further determined by Western blot analysis with a panel of 48 HIV-2-positive and 22 HIV-2-negative serum samples (1:1,000 dilution) from Senegal. Crude lysates of the recombinant proteins ST7-8, ST9-10, ST7-10, ST13-14, and ST0.95 were similarly analyzed. Among the seven recombinant proteins studied, ST11-12 and ST15-16 were reactive with 46 of 48 (95.8%) and 47 of 48 (97.9%) HIV-2-positive serum samples, respectively (Table 2). A false-positive result was noted for one serum sample against the recombinant protein ST15-16. Less than 10% of the HIV-2-positive serum samples tested had reactivity to the recombinant proteins ST9-10, ST7-10, and ST13-14; no reactivity was detectable with the recombinant proteins ST7-8 and ST0.95. A similar analysis was performed with HIV-1-positive serum samples from 20 donors in the United States to determine whether these HIV-2 recombinant proteins could be used to differentiate between HIV-1 and HIV-2 infections. One of the 20 serum samples was found to have weak reactivity to the recombinant protein ST15-16, while none reacted to the recombinant protein ST11-12.

Results obtained with HIV-2-positive, HIV-2-negative, and HIV-1-positive serum samples tested against ST11-12 and ST15-16 are shown in Fig. 2.

To further test whether the immunoreactive domains of the HIV-2 envelope are also present in sera from different

A. ST11-12



B. ST15-16

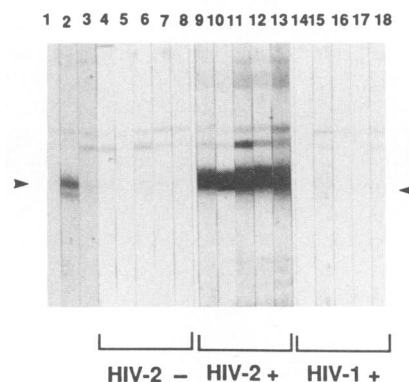


FIG. 2. Western blot analysis of the recombinant proteins ST11-12 (29 kDa) (A) and ST15-16 (30 kDa) (B). Sizes of the proteins are indicated by arrowheads. Lanes 1, anti-*ras* preimmune serum; lanes 2, anti-*ras* immune serum; lanes 3, normal human serum; lanes 4 through 8, representative HIV-2-negative sera; lanes 9 through 13, HIV-2-positive sera; lanes 14 through 18, HIV-1-positive sera. Lanes representing HIV-2-negative, HIV-2-positive, and HIV-1-positive sera were selected from separate gels analyzed simultaneously (1:1,000 dilution), each with proper controls. Visualization of the recombinant proteins was performed by secondary staining with biotinylated goat antihuman serum and then with avidin-horseradish peroxidase complex (Amersham Corp., Arlington Heights, Ill.). The final color was developed by 3,3'-diaminobenzidine.

TABLE 3. Immunoreactivity to ST11-12 and ST15-16 of serum samples from various West African countries (23)

Source	No. of reactive samples/no. tested		
	HIV-2 positive		HIV-2 negative (ST11-12 or ST15-16)
	ST11-12	ST15-16	
Benin	4/4	4/4	1/2
Burkina Faso	15/22	18/22	0/4
Guinea	4/6	5/6	
Guinea-Bissau	13/13	13/13	0/11
Ivory Coast	3/3	3/3	0/1
Total	39/48	43/48	1/18

geographical regions of West Africa, samples (23) from Benin, Burkina Faso, Guinea, Guinea-Bissau, and the Ivory Coast were subsequently analyzed by Western blot. Comparable results were obtained (Table 3); 81.3% (39 of 48) of HIV-2-positive serum samples tested positive on ST11-12 and 89.6% (43 of 48) were positive on ST15-16. The differences in immunoreactivity were mainly in sera from Burkina Faso. One of the negative serum samples from Benin was weakly positive. The results further attest to the existence of two strongly immunoreactive regions in both the extracellular and the transmembrane domains of the HIV-2 envelope.

From the results of Western blot analysis, we identified two highly immunogenic domains in the HIV-2_{ST} envelope. The first is the recombinant protein ST15-16, covering the amino terminus of the transmembrane glycoprotein gp36. This finding is consistent with results for HIV-2_{ROD} described by Schulz et al. (42) and for HIV-2_{NIH-z} described by Zuber et al. (50) (see the sequence comparison in Table 4 for the respective corresponding sequences in each strain). Comparatively, linear epitopes in this part of the HIV-2 envelope share certain similarities with those of the HIV-1 envelope. In the study by Samuel et al. (40), nine recombinant peptides derived from the HIV-1_{BH10} envelope were expressed in bacteria and their antigenicities were determined by screening against a panel of HIV-1-positive human sera. The recombinant protein 566 derived from the NH₂-terminal region of gp41 (aa 548 to 736) reacted strongly with the reference sera. This region corresponds to ST15-16 (aa 541 to 732) of the HIV-2_{ST} envelope transmembrane sequence (Table 4) and is useful for type-specific detection of HIV-1 infections (40). Thus, the presence of a highly immunogenic domain in the amino terminus is a characteristic common to the transmembrane glycoproteins of both HIV-1 and HIV-2.

The second highly immunogenic domain is localized in the middle of the extracellular glycoprotein, gp120, represented by ST11-12. It is worth noting that the coding region for ST11-12 is moderately divergent among HIV-2 strains (Fig. 3), more so at the carboxyl end of the peptide, and corresponds to the highly immunogenic V3 region of HIV-1 (Table 4) (28). Our finding that more than 81% of all HIV-2-positive serum samples reacted with this peptide contrasts with the low immunoreactivity reported for the recombinant proteins encoded by corresponding sequences in either HIV-2_{ROD} (42) or HIV-2_{NIH-z} (50). One explanation for this discrepancy is that most of the HIV-2-positive sera that we studied may have come from people exposed to HIV-2 strains with higher sequence homology to HIV-2_{ST} than to HIV-2_{ROD} or HIV-2_{NIH-z}. However, we do not have the sequence information to substantiate this hypothesis at present. Alternatively, ST11-12 may comprise linear epitopes which are structurally and functionally more antigenic than the corresponding regions in HIV-2_{ROD} or HIV-2_{NIH-z}. Therefore, antibodies present in the infected immune sera may recognize the HIV-2_{ST} antigen better. Further comparative antigenicity studies of different strains of HIV-2 are needed to confirm this hypothesis.

Serological cross-reaction between HIV-1 and HIV-2 has been well documented (4, 44, 46, 47). In a recent study by Böttiger et al. (4), cross-reactions were observed by Western blot in 10% and by radioimmunoprecipitation analysis in 40% of the HIV-1-positive serum samples and by Western blot in 29% and by radioimmunoprecipitation analysis in 48% of the HIV-2-positive serum samples. Similarly, Syu et al. (44) reported that approximately 50% of HIV-1 serum samples cross-reacted with HIV-2 by radioimmunoprecipitation analysis. This high degree of serological cross-reactivity between HIV-1 and HIV-2 may be attributed to the preservation of common epitopes in its nascent conformation by the envelope glycoprotein of both types of virus when whole virus is used as antigen in the analysis. Such preservation of common epitopes may be absent in the recombinant peptides. So far, synthetic peptides corresponding to the immunodominant region in the amino terminus of the transmembrane glycoprotein of HIV-2_{ROD} and SIV_{mac} are the only serologic markers known to be able to distinguish HIV-1 from HIV-2 infections (16, 21, 33). However, 2.5 to 6.1% of the HIV-1-positive serum samples studied also reacted with these peptides (15). In contrast, our finding that none of the HIV-1-positive serum samples studied had cross-reactivity to the HIV-2 recombinant protein ST11-12 indicates that this highly immunogenic domain in the extracellular glycoprotein of HIV-2_{ST} has the potential

TABLE 4. Viral sequences corresponding to the amino acid sequences of the expression clones in the HIV-2_{ST} envelope^a

Clone fragment	Position in:					
	ST	ROD	NIH-z	SBL/ISY	SIVmm251	HXB2
ST7-8	1-114	1-120	1-113	1-114	1-127	12-131
ST9-10	119-219	125-225	116-215	117-216	132-233	132-216
ST11-12 ^b	224-380	230-388	220-378	221-378	238-393	221-375
ST13-14	386-519	394-525	384-516	384-517	399-541	380-526
ST15-16 ^b	524-763	530-769	522-760	522-761	546-785	531-760
ST0.95	769-859	775-858	766-859	767-846	791-881	786-856

^a Sequences were compared by the DNASTAR DNA analysis program by using the Needleman-Wunsch algorithm for amino acid sequence comparison with a gap penalty of 4 and the Wilbur-Lipman algorithm for nucleic acid sequence comparison with a K-tuple of 3 and a gap of 3. Sequences were retrieved from GenBank 1990 release.

^b ST11-12 and ST15-16 are regions containing the most immunogenic epitopes.

	224v	230v	240v	250v	260v	270v	280v
ENV2ST	PGFALLRCNDTNYSGFEPNC	SKVVAATCTRM	MTQTSTWFGFNGTRA	ENRTYIYWHGRDN			
ENV2GH1	Y	K	S				
ENV2ISY	V		S	P	L		
ENV2NIHZ		A					K
ENV2ROD	Y	A	S				
ENV2BEN			K T	S			

	290v	300v	310v	320v	330v	340v	350v
ENV2ST	RTIISLNKFPYNLTVHCKRPGNKTVVPITLMSGLVFHS--QP-INRRPRQAWCWFKEGWEKAMKEVKLTAKHPR						
ENV2GH1	Y	SI			T	K R	Q Q I
ENV2ISY	Y	IL R E		RR	KI KK	R R	Q Q V
ENV2NIHZ	N	M	L F	FK	V KK	E Q	Q E
ENV2ROD	Y	SL	I KQ M	H	HY K	K D	Q E
ENV2BEN	Y	MR	L		T	R G R R	Q Q VQ

	360v	370v	380v
ENV2ST	YKGT-NDTEKIRFIAPGERSDPEVAYM		
ENV2GH1	KN N TK	RG	
ENV2ISY	N N T	EKD	
ENV2NIHZ	NRSR N K K	RG	T
ENV2ROD	R RN S A	KG	
ENV2BEN	I G N TK	AG	

FIG. 3. ST11-12 amino acid sequence homology among the HIV-2 strains. Sequences were compared by using the DNASTAR DNA analysis package (DNASTAR, Inc., Madison, Wis.) as described in Table 4, footnote a.

to be used as another type-specific serologic marker for HIV-2 infection.

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